Remarks

Claims 38-61 and 64 are pending.

The present invention is directed to polyesters made in cells which express a PHA synthase, which have been genetically engineered to express a recombinant fatty acid:acyl CoA transferase. The claims further define other modifications of the cells to express other enzymes, or selection of the cell to express other enzymes. Claim 38 was amended in order to clarify the expression of the synthase and transferase under the control of a single promoter (i.e. co-expression). Claims 52-56 have been amended to clarify that nucleic acid segments are being expressed.

Rejection Under 35 U.S.C. § 102

Claims 38-42, 44-61 and 64 were rejected under 35 U.S.C. § 102(e) as being anticipated by U.S. Patent No. 6,117,658 to Dennis *et al* ("Dennis"). Applicants respectfully traverse this rejection.

Enclosed is an executed Declaration under 37 C.F.R. § 1.131 (4 copies; each signed by a separate inventor) confirming that the above-identified applicants conceived and reduced to practice the claimed methods prior to the filing of application U.S.S.N. 09/023,029 filed February 12, 1998 (issued as U.S. Patent 6,117,658). Enclosed are exhibits copies of a FEMS Microbiology reference (labeled Exhibit 2) and letter transmitting the FEMS manuscript for peer review (labeled Exhibit 1). The signed letter is dated January 21, 1997, clearly showing that the

claimed methods were conceived and reduced to practice at least 3 weeks prior to the filing of U.S. Provisional Application No. 60/037,758.

Rejection Under 35 U.S.C. § 103

Claims 38-39 and 41-43 were rejected under 35 U.S.C. § 103(a) as being unpatentable over U.S. Patent No. 6,117,658 to Dennis *et al* ("Dennis"), in view of U.S. Patent No. 5,512,468 to Greener *et al.* ("Greener"). Applicants respectfully traverse this rejection.

Dennis

An executed Declaration under 37 C.F.R. § 1.131 confirming that the above-identified applicants conceived and reduced to practice the claimed methods prior to the filing of application U.S.S.N. 09/023,029 filed February 12, 1998 (issued as U.S. Patent 6,117,658) is enclosed.

Greener

With respect to Greener, the Examiner asserts that there is motivation to arrive at the claimed method in certain types of *E. coli* cells. This assertion is based on the idea that transformation is somehow functionally linked to the production of enzymes. Column 1, lines 16-18, of Greener clearly defines the term "transformation". The functional expression of a gene of interest is dependent upon events beyond the "unidirectional transfer and incorporation of foreign DNA by the cell". While nucleic acid uptake by the cell is required for the production of protein encoded by the DNA, proper transcriptional, translational, and post-translational apparatuses and mechanisms are required for the functional, and increased, production of the

enzymes and enzymatic activity encoded in the transforming nucleic acid. Therefore while the expression of functional alpha amylase or LacZ had been shown in XL-1 Blue cells (per the Greener disclosure), this is not predictive of the successful preparation of a polyester from recombinant cells expressing PHA synthase protein and fatty acid:acyl-coenzyme A transferase protein.

However, even if there were a disclosure of each of the claimed elements, and the motivation to combine, with an expectation of success (which we do not concede), the method as defined by the amended claims produces unexpected results. The claims have been amended to recite that the synthase and transferase are on a single element. This results in substantially higher production of polymer than the introduction of the synthase and transferase on separate nucleotide molecules, as described by Dennis.

Referring to the examples, several plasmids were made as described in example 1:
Plasmids pKSSE5.3 and pSKSE5.3 contain the PHA synthase and transferase
Plasmids pKSSA35 and pSKSA35 contain the PHA synthase and thiolase
Plasmids pKSAE18 and pSKAE18 contain transferase only.

Example 2 demonstrates that putting both genes adjacent and co-linear with the promoter significantly increases polymer yield. This finding is completely unexpected and could not have been obvious from any of the cited art, alone or in combination.

In view of the foregoing discussion related to Greener, the applicants submit that Greener alone does not make obvious the claimed subject matter.

Allowance of claims 38-61 is respectfully solicited.

Respectfully submitted,

Patrea L. Pabst
Reg. No. 31,284

Date: June 27, 2003

HOLLAND & KNIGHT LLP
One Atlantic Center, Suite 2000
1201 West Peachtree Street
Atlanta, Georgia 30309-3400
(404) 817-8473
(404) 817-8588 (Fax)

CERTIFICATE OF MAILING 37 C.F.R. 1.8(a)

I hereby certify that this paper, along with any paper referred to as being attached or enclosed, is being deposited with the United States Postal Service on the date shown below with sufficient postage as first-class mail in an envelope addressed to the Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Aisha Wyatt

Date: June 27, 2003

In the Claims

38. (Twice Amended) A method for the preparation of a polyester, comprising the steps of: culturing recombinant cells under conditions suitable for the production of the polyester, wherein the recombinant cells ~~co-express a polyhydroxyalkanoic acid synthase protein and~~ have been genetically engineered to express a polyhydroxyalkanoic acid synthase and a fatty acid:acyl-coenzyme A transferase protein under the control of a single promoter.

39. (original) The method of claim 38, wherein the cell is a plant cell, mammalian cell, insect cell, fungal cell, or bacterial cell.

40. (original) The method of claim 39, wherein the cell is a plant cell.

41. (original) The method of claim 39, wherein the cell is a bacterial cell.

42. (original) The method of claim 41, wherein the cell is *Escherichia coli.*

43. (original) The method of claim 42, wherein the bacterial cell is *Escherichia coli* strain XL1-Blue.

44. (original) The method of claim 38, wherein the polyhydroxyalkanoic acid synthase protein is a polyhydroxyalkanoic acid synthase protein from *Alcaligenes eutrophus.*

45. (original) The method of claim 44, wherein the *Alcaligenes eutrophus* polyhydroxyalkanoic acid synthase protein is encoded by the *Alcaligenes eutrophus* polyhydroxyalkanoic acid synthase structural gene.

46. (original) The method of claim 38, wherein the fatty acid:acyl-coenzyme A transferase protein is a 4-hydroxybutyrate:acyl-coenzyme A transferase protein.

47. (original) The method of claim 46, wherein the 4-hydroxybutyrate:acyl-coenzyme A transferase protein is a *Clostridium kluyveri* 4-hydroxybutyrate:acyl-coenzyme A transferase protein.

48. (original) The method of claim 47, wherein the *Clostridium kluyveri* 4-hydroxybutyrate:acyl-coenzyme A transferase protein is encoded by *Clostridium kluyveri orfZ* 4-hydroxybutyrate:acyl-coenzyme A transferase structural gene.

49. (original) The method of claim 38, wherein the culture contains glucose.

50. (Twice amended) The method of claim 38, wherein the culture contains materials selected from the group consisting of 4-hydroxybutyric acid, the sodium salt of 4-hydroxybutyric aicd, γ-butyrolactone, 1,4-butanediol, 4-hydroxyvaleric acid, γ-valerolactone, 1,4-pentanediol, 3-hydroxybutyric acid, the sodium salt of 3-hydroxybutyric acid, a hydroxypropionic acid, a hydroxybutyric acid, a hydroxyvaleric acid, a hydroxycaproic acid, a hyroxyheptanoic acid, a hydroxyoctanoic acid, a hydroxydecanoic acid, γ-caprolactone, γ-heptanolactone, γ-octanolactone, ~~or~~ γ-decanolactone.

D2

51. (original) The method of claim 38, wherein the culture contains molecular oxygen.

52. (Twice Amended) The method of claim 38, wherein the cell ~~is genetically engineered to express~~ expresses a heterologous nucleic acid segment encoding a protein capable of hydrolyzing a lactone to the corresponding hydroxyalkanoic acid.

D3

53. (Twice Amended) The method of claim 38, wherein the cell ~~is genetically engineered to express~~ expresses ~~a~~ heterologous nucleic acid segments encoding 2-oxyglutarate decarboxylase protein and a heterologous 4-hydroxybutyrate dehydrogenase protein.

54. (Twice amended) The method of claim 38, wherein the cell ~~is genetically engineered to express~~ expresses a heterologous nucleic acid segment encoding a protein selected from the group consisting of a 2-methylcitrate synthase protein, a 2-methylcitrate dehydratase protein, 2-methylisocitrate dehydratase protein, 2-methylisocitrate lyase protein, a succinate:aceyl-CoA transferase protein, a succinate-semialdehyde dehydrogenase protein, and a 4-hydroxybutyrate dehydrogenase protein.

55. (Twice amended) The method of claim 38, wherein the cell ~~is genetically engineered to express~~ expresses ~~a~~ nucleic acid segments encoding succinate-semialdehyde dehydrogenase protein, and a 4-hydroxybutyrate dehydrogenase protein.

56. (Twice amended) The method of claim 38, wherein the cell ~~is genetically engineered to express~~ expresses ~~a~~ nucleic acid segments encoding 2-methylisocitrate dehydratase protein, a 2-methylisocitrate dehydratase protein, a 2-methylisocitrate dehydratase protein, a 2-methylisocitrate lyase protein, a succinate:acetyl-CoA transferase protein, a succinate-semialdehyde dehydrogenase protein, and a 4-hydroxybutyrate dehydrogenase protein.

57. (original) The method of claim 38, wherein the polyester is a homopolyester.

58. (original) The method of claim 57, wherein the homopolyester is poly(4-hydroxybutyric acid).

59. (original) The method of claim 57, wherein the homopolyester is poly(3-hydroxybutyric acid).

60. (original) The method of claim 38, wherein the polyester is a copolyester.

61. (original) The method of claim 60, wherein the copolyester is poly(3-hydroxybutyric acid –co-4-hydroxybutyric acid).

D4

64. (newly added in the response mailed on February 22, 2002) The method of claim 38 further comprising separating the polyester from the recombinant cells.